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METHODS OF SENDING SPECIMENS OF WATER¹

BY JACK J. HINMAN, JR.²

In order that a water supply may be examined fairly, it is of primary importance that a true sample be submitted to the analyst for examination. Anything which might result in the introduction of foreign material, especially bacteria, may easily result in improper findings which would usually be to the disadvantage of the supply.

The first requisite for the collection of the sample is a properly prepared bottle. Any bottle or jug will not do. It must be chemically clean, and if there is a bacteriological examination to be made, it must be sterile. Any cleaning solution used should be thoroughly removed, since many such solutions are germicidal in character. The sterilization of the bottle should be accomplished by boiling in water, steaming or by the use of dry heat. To attempt to sterilize a bottle by means of chemical disinfectants may lead to erroneous results unless the greatest care is taken to rinse out the last traces of the chemical; in which case the repeated rinsings may defeat the sterilization by introducing contaminating material. The bottle should be closed with a glass stopper, because cork stoppers are difficult to sterilize by the means usually at hand outside of the laboratory, and have the additional disadvantage of yielding various substances to the water from the many cracks and crevices. The use of corncobs, rolled paper and such substances is naturally to be avoided.

Jugs should never be used because the roughness of their interiors is likely to cause adhering materials to be much more difficult to remove. It is also impossible to see whether or not the jug is clean. Supposedly clean new jugs frequently contain straw and other similar substances. Many jugs are glazed by the use of salt which may add to the chlorine content of the water.'

¹ Read at the first annual meeting Iowa Section, American Water Works Association, at Iowa City, December 3, 1915.

² Senior water bacteriologist and chemist, Laboratories for the State Board of Health, Department of Pathology and Bacteriology State University of Iowa, Iowa City.

It is best in all cases to send to the examining laboratory for the collecting bottles because any such laboratory will be found equipped with suitable apparatus for the cleaning and sterilization of the sample bottles. The laboratory should provide some means of sealing sterile glassware and you should receive the bottle with the seals intact, thus assuring you that they are in condition for use. Having received the sterile bottles the collection of the water must be carefully made. It is easy to set at naught the pains that have been taken to furnish a clean sterile container. It is usually a waste of time and money to entrust the collection of samples of water to an uninstructed workman. The collector should be familiar with the usual precautions to be observed in taking a sample of water, since everyone does not appreciate the ease with which bacteria may be introduced or a measurable quantity of foreign matter added. When chemical tests are made to one part in a hundred or a thousand million in routine examination and bacteria are counted, the ubiquity of bacteria and the great influence of an almost imperceptible amount of dirt must be taken into consideration.

The usual precautions are as follows:

1. Allow the water to flow until the water which has stood in the pipe has wasted, because since it has stood in a more or less warm place for some time, the bacteria have probably increased in number and chemical changes have gone on. If the sample is taken from a street hydrant or similar source an accumulation of iron will need to be flushed out.
2. Remove the cover from the top of the bottle, holding it in such a way that the inside of it is not soiled by contact with the hand or any other object. Open the bottle while the water is running; fill it nearly full, rinse, refill; rinse the ground part of the stopper and insert the stopper. Take great care throughout that the stopper, the inside of the neck of the bottle and the water collected are kept from contact with the outside of the faucet, the hand, a cloth or any other object.
3. Protect the top of the bottle from dust and other contaminating substances by replacing its cover of tin-foil or cloth. When a sample of water has been properly collected in a proper sort of container, the manner of shipment to the laboratory must be considered. Chemical and bacterial changes take place with varying rapidity, depending on the character of the water, the temperature at which it is kept, the exposure to light, etc. To secure the best results,

that is, to deliver it to the laboratory in as nearly unchanged condition as possible, the time consumed in transit should be short and the temperature of the water should be kept low. The low temperature retards the multiplication of bacteria and minimizes the chemical change.

For some time the water laboratory of the Iowa State Board of Health has been using 1 gallon glass stoppered bottles for the collection of water samples. The bottle is slipped into a felt-lined case, open at the top; it fits tightly into the case and the felt lining serves to protect the bottle from breakage and to protect its contents to a certain extent from extremes of temperature. The case is left open at the top so that the expressmen can see what they are handling and use suitable caution. Concerning the breakage of bottles in shipment it is only fair to state that our breakage has been less than 1 per cent.

The bottle is prepared for use by cleaning with a strong solution of potassium bichromate and sulphuric acid, as directed in the *Standard Methods of Water Analysis*, and then sterilized by steam. A sterilized muslin cloth is tied over the top by a stout cord, the ends of which are then sealed upon the top of the stopper by an official wax seal. Owing to the fact that the bottles are necessarily of a rather thick glass, they are often cracked during sterilization. This occasions most of our breakage of bottles, which is a factor of consequence, since the bottles cost about 80 cents a piece.

The gallon-bottle outfit was adopted when the laboratory was first established because the investigations of Whipple and others (as well as some work in our own laboratory) had shown that chemical and bacterial changes are less in a large bottle of water than in a small bottle of the same water, given the same temperature conditions.

A long series of experiments carried out during the summer months has shown us that, while we are able to get bacteriological results of considerable diagnostic value from samples so collected, the bacterial counts are not rigidly exact enough to be of great value in checking up the operation of a water works filter plant. For this purpose, we have used a smaller bottle packed in a case which may be iced. Although the bacterial examination is the chief requirement in such cases, it may often be that a chemical examination is required as well. To collect the large sample as well as a small one is inconvenient and the extra expressage is not an inconsiderable item.

For field work the large bottle is unhandy. It is difficult to collect a sample from streams or ponds especially if they be shallow. Although the bottle contains 3700 to 3800 cc. only about 800 cc. of water are used in the routine procedure. The excess is of value only as it helps to retard the changes in the sample.

Penniman and Enslow of the Maryland State Board of Health have recently reported on their successful use of a 1 pint sample for chemical analysis. This container was not intended to furnish a bacteriological sample as well. Since only a few more cubic centimeters are required for the ordinary bacterial counts and fermentation tubes, it occurred to us that we might use a similar sized bottle and send the sample packed in ice in a case slightly smaller than the old bacteriological case which we had been using. The ice packing should serve to retard chemical and bacterial changes even more than the use of such a container as our old one. Under such conditions it is necessary to collect only a small extra quantity of water to allow for accidents, and for rinsing of the measuring vessels.

By reducing the quantity of water used in the ammonia determinations from 500 cc. to 100 cc. the amount of water collected may be reduced to 500 cc. and yet leave a margin of 100 cc. to provide for an emergency. Penniman and Enslow claimed to be able to do this without reducing the accuracy of the method. Our preliminary work has verified their results in a general way, although we find that it is more difficult to read low ammonias. This is due to the fact that our first tube of 25 cc. of distillate represents a concentration of most of the ammonia in an amount of water equal to one-fourth the volume of water used in the determination, while in the old method, we have a concentration to one-tenth the volume.

Penniman and Enslow used short shell vials for Nessler jars which they graduated themselves. We have used homemade Nessler jars blown out of $\frac{1}{2}$ -inch glass tube. These provide a longer column of liquid. We have used small Kjeldahl flasks instead of small retorts such as were used by Penniman and Enslow. Our routine chemical examination includes nitrogen as free and albuminoid ammonias, nitrites and nitrates and chlorine. It is not necessary to reduce the quantities of water taken for the other tests.

The new case is in the form of a box about $8\frac{1}{2}$ by $8\frac{1}{2}$ by 11 inches furnished with hinged cover, hasp and handle. The inside dimensions are 7 by 7 by $9\frac{1}{2}$ inches. It is lined with galvanized iron. The bottle is enclosed in a cylindrical galvanized box 4 inches in

diameter and 9 inches high which on account of its dimensions can not fall over in the box and fill with water produced from the melting ice. A lead seal securing the hasp of the box insures the sterility of the bottle.

It requires about 6 pounds of ice to fill the container properly. A few ounces of excelsior or saw dust should be added with the ice so that the bottle may carry well and the ice be kept from rapid melting. Excelsior is cleaner to handle than sawdust but it does not protect the ice as well.

Most of our samples reach us about twenty-four hours after collection. Our experiments at the laboratory, using the very inferior natural ice which has been available, show that this container will keep the temperature of the sample for a period of forty-eight hours several degrees below the surrounding temperature even in hot weather.

The box, metal fittings and bottle complete cost in the neighborhood of \$1.75 or \$2